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Vol. XLIV *April, 1923.* *No. 4.*

BIOLOGICAL BULLETIN

MOTOR REACTIONS OF THE FRESH-WATER SPONGE, *EPHYDATIA FLUVIATILIS*.[1]

GEORGE T. McNAIR,

HULL ZOÖLOGICAL LABORATORIES, THE UNIVERSITY OF CHICAGO.

Parker's work on *Stylotella heliophila* ('10) has suggested the possibilities of similar experiments with fresh-water sponges and a comparison of the reactions of the two types.

Aside from Parker's monograph practically nothing has been published on the motor reactions of sponges. Dr. R. E. Grant (1825–'26) published a series of papers on the "Structure and Functions of Sponges," which are of historical interest only. He quotes an earlier paper (Ellis and Knight, *Transactions of the Royal Society of London*, 1765), in which these men stated that they had seen the orifices on the surfaces of sponges contract and dilate themselves. Grant studied a large number of salt-water species, both in their natural habitat and in the laboratory. He was the first to notice and accurately describe the currents of water coming from the fecal orifices (oscula) and spoke of them as mysterious currents because he could not learn what caused them. He irritated the orifices and other tissues with corrosive acids, red hot wires, etc., but under no circumstances did he see the osculum closed and erroneously concluded that there was a complete lack of irritability and contractility, attributing the statements of Ellis and Knight concerning the "systole and diastole of the fecal orifices" to "some optical deception a little assisted by the imagination."

Parker made two types of experiments on *Stylotella*, noting

[1] The problem was suggested to me by Dr. W. J. Crozier. It is with great pleasure that I acknowledge my indebtedness to him and also to Professors C. M. Child and W. C. Allee for their helpful suggestions and criticisms.

the responses of the oscula, ostia, and choanocytes first to mechanical and then to chemical stimuli. He studied the reactions of the sponge to flowing water, injuries, and cuts. Likewise he studied the effects which ether, chloroform, cocaine, strychnine, and atropine produced upon the sponge and compared his results with the known effects of these drugs on smooth muscle and on ciliated tissue.

Ephydatia fluviatilis (L.), the fresh-water sponge with which the experiments described in this paper were made, differs so greatly in structure from *Stylotella* that it was impossible to duplicate many of Parker's experiments. The responses of the oscula were watched to determine the effects of various mechanical stimuli such as injuries, electrical stimulation, and changes in temperature. The purpose was to determine the effects of such stimuli and the rate of possible transmission of these effects from one part of the body to another.

All the sponges used in the following experiments were collected in small ponds along the railroad tracks in the neighborhood of Hammond and Buffington, Indiana. They were carried to the laboratory in fruit jars and then transferred to large jars of slowly running water.

The ponds from which the material was collected[1] were shallow, often almost filled with bulrushes. The bottoms were covered with mud and cinders. The sponges were, for the most part, growing on the under side of old railroad ties floating in the water and were more or less cushion-like in form and without much branching. Some, however, were found growing on the submerged parts of plants or on leaves, but here their form was more spreading, following that of the body to which they were attached.

The body of the sponge is dotted all over with very small dermal pores or ostia. The oscula are relatively prominent little chimneys of dermal membrane, standing our from almost any point on the suface. Bundles of smooth, pointed, and almost straight silicious spicules make up the skeleton. The internal canals of the body meander irregularily between these bundles of spicules. The dermal membrane, which completely envelops

[1] See Shelford, "Animal Communities in Temperate America," Chapter VIII.

the body, is held up in tent-like elevations by the protruding ends of the spicules.

The oscular chimneys seem to be kept open by the pressure of the currents of water passing out through them. They are continuously changing their form. At times one will be long and slender; again it will be shorter and much thicker or even dome-like, the base being as much as three or four times the height. In instances of the first type the mouth of the chimney will probably be open almost to the width of the diameter, although these mouths are frequently seen to be nearly closed. In the type last mentioned they are usually small.

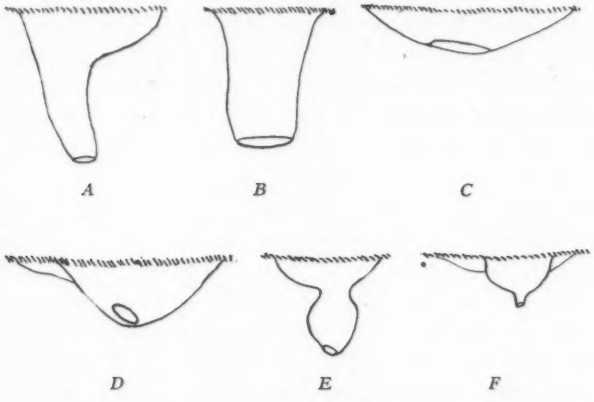

FIG. 1. Various forms in which the same oscular chimney was observed.

These variations in shape lead to the belief that the entire chimney contains sphincter-like bands. If all such bands were about equally contracted the result would be a long slender chimney, Fig. 1, *A* and *B*. If only those at the end contracted and the others relaxed, the result would be the dome-like shape, Fig. 1, *C* and *D*. Several instances of a contraction of the terminal sphincter and also of the chimney near its base, with a relaxation between, were noticed. The result was a sort of globe-like chimney attached to the sponge body by a narrow neck, Fig. 1, *E*. The chimneys also seem capable of a decided shortening without much change in diameter. This may be

brought about by a contraction of longitudinal fibers, but it could not be definitely demonstrated.[1]

Effects of Currents of Water on the Osculum.

Potts ('87) makes the statement that he found *E. fluviatilis* in both quiet and running water. It seems to grow best in ponds of quiet water, but very fine specimens are found on the under side of rocks in rapids or at the base of water falls where the river current is the swiftest.

To get an idea of the effects of water currents, a few sponges were placed in a flat dish containing about a quart of water and left over night. The next morning the oscula were still expanded, and, by watching the movements of tiny particles of suspended material in the water, steady currents could be seen flowing through them. This test was repeated with the same results. It was noticed, however, that if the sponges were left in such a dish of unchanged water much longer than a day they gradually became less active and in many cases soon died.

To determine the effect of a swift current, a glass tube was connected with the water tank and placed in one side of a dish containing the sponges. The water was turned on at full force and left running for about four hours. After transferring the sponges to a watch glass for observation (without taking them from the water) it was found that the entire chimney had contracted until it was almost flat, but that the mouth of the osculum was wide open and currents of water were coming out. The following illustration shows the extent of the contraction of the chimney.

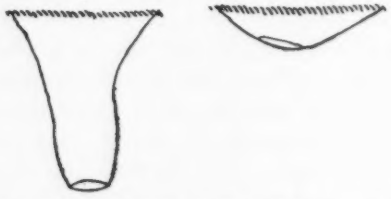

Fig. 2. A chimney before and after a strong current of water had been applied, showing the extent of the contraction.

[1] See, E. A. Minchin, Lankester's "Treatise on Zoölogy," p. 44.

Within thirty minutes after taking the sponges out of the swift current the chimney had again extended to about half of its original length. The osculum was slightly contracted.

The current had caused the sponges to roll and tumble over the bottom of the dish to some extent. To find out if this had been the cause of the shortening of the chimneys, the sponges were placed in watch glasses which, in turn, were placed within the larger dish. When the water was turned on, the force of the current against the curvature of the watch glass held the sponges in place. This time a swift current flowed over the sponges but they did not roll about. Within twenty minutes the chimneys had flattened out as before.

The sponges were now placed in a large jar in which a slow current of water was kept going. After being there for a few hours the chimneys were found to be well extended and with strong currents coming out through them.

Neither the absence of currents nor the presence of strong currents causes the osculum to close although the latter does cause a general shortening of the oscular chimney. In small quantities of water, however, the sponges soon became less active and in time died; the smaller the quantity of water the sooner the changes could be noticed. This effect could not have been due to the absence of the mechanical stimulus of flowing water, because if placed in a large tank of quiet water the sponges will live for a long time. Nor could it have been caused by lack of oxygen, because the dishes which were used were large and shallow so that the water was well exposed to the air. It must have been produced then by self-poisoning from the products of metabolism that accumulated in the small quantity of water. The greater general vitality of the sponges when placed in slow running water or in large tanks of quiet water is accounted for by the fact that these products were removed as they were thrown off by the sponges or were diffused through the large volume of water.

These results are entirely different from those obtained by Parker from somewhat similar tests with *Stylotella*. When he transferred *Stylotella* from the natural habitat to tanks of quiet sea water, the oscula invariably closed within ten minutes and

did not reopen until they were placed in running water. He was able to prove that the mechanical stimulus of the currents of water on the outlet tip of the finger where the osculum was located, caused it to open. The oscular closure seems to be a protective adaptation of *Stylotella*, which is a shallow salt-water species frequently left exposed to the air when the tide is out. Since the fresh-water sponge is never exposed to such tidal conditions, one would not expect to get the same reactions.

Brushing.

Rubbing the sides of the chimney with a needle, prodding it gently, or even inserting the needle into the mouth of the osculum and rubbing the inside of the chimney, caused no noticeable reaction; however, if the needle were carefully rubbed around the edge of the mouth of the osculum, an immediate contraction of the orifice would follow and it would continue to contract for about three minutes. Following the contraction of the sphincter at the orifice a wave of contraction would travel down the chimney although the amount of contraction would be less than at the orifice. On a chimney of 1.5 mm. length it would require about six seconds for this wave to run from the tip to the base of the osculum. In the next three or four minutes it would again expand to its original size. The edge of the mouth of the osculum, therefore, seems to be more sensitive than the rest of the chimney. Concerning the reactions of *Stylotella*, Parker states that stroking an open osculum with a bristle or brush does not cause it to close, but if it is closed, this treatment may at rare intervals induce it to open.

Sharp Blow.

Several times while getting the electrodes into position to work with the inductorium one of the wires of the electrode would catch on a spicule and, springing loose, strike an oscular chimney. Each time the entire chimney would shrivel up immediately in a more or less collapsed condition, but within twenty or thirty minutes it would be open and functioning as before.

Cutting.

Cutting into the body seemed to have no effect in the way of induced movements. With a small scalpel an incision was made

into the body of the sponge 3 mm. from the base of the chimney without causing any change in the rate of the currents or in the size of the chimney. Another cut right at the base of the chimney caused it to close. It did not reopen for two days. This cut was so near the chimney, however, that the closing was probably due to cutting into the chimney cavity and when this had healed over the chimney again expanded. The result of such injuries differs from the effect of similar ones on *Stylotella*, where a cut from 3 mm. to 5 mm. from the osculum was found to cause closure within nine minutes.

Pin Sticking.

Sticking a needle into the flesh of the sponge at a distance of 2 mm. from the osculum caused no noticeable effect on the osculum. The same type of injury 5 mm. from the osculum of *Stylotella* resulted in the osculum closing within ten minutes and remaining closed for several hours.

Exposure to the Air.

A sponge was lifted from the water and held in the air for three minutes. A large chimney was noticeable before, but could not be found after placing the sponge back in the water; neither could it be found at any later time.

Thermal Responses.

The average temperature of the water in the tanks in which the sponges were kept was about 27° C. Eight of the sponges which had well developed oscular chimneys and from which strong currents of water were flowing were placed in dishes in a refrigerator which had a uniform temperature of 5° C. and left until the water had been cooled to 7° C. They were examined at regular intervals. At 16° there was no noticeable change in any of the sponges. At 11° all had a shriveled appearance, some of the oscula were open slightly, and very slow currents coming from them were barely visible. At 10° no oscula could be found, and at 7° the dermal membrane had completely shrunk in over the body of all the sponges and no sign of activity could be detected. The dishes were transferred to an incubator and

observations were made until the temperature of the water had reached 40° C. At 24° the dermal membranes had taken on their usual appearance, the oscula were somewhat expanded and currents were issuing from them. At 34° the chimneys were more extended and stronger currents were coming from them than at any previous time. At 37° they were still expanded but were slightly flabby and the currents were weaker. At 40° the oscula had all disappeared, the dermal membranes were completely shriveled. None of the sponges recovered after being raised to the temperature of 40°.

Comparing these results with those obtained by Parker, it will be seen that the salt-water sponge is apparently less affected by temperature conditions than is the fresh-water species. With *Stylotella* at between 9° and 10° the oscula and ostia remained open and the only effect seemed to be that the currents became slow. At 40° there was a slight constriction of the osculum and the currents gradually became slower and stopped. At 45° there was a flabby contraction of the oscula and the currents ceased abruptly. They did not recover after being raised to the temperature of 45°.

Light.

Neither full sunlight nor complete darkness seemed to have any effect on *E. fluviatilis*. One large, healthy specimen was placed in a dark cupboard and left there four days. At the end of this time the green color due to contained algæ had faded, but the activity of the sponge in producing currents had not diminished in any way.

Electrical Stimulation.

By using an inductorium with electrodes of fine platinum wires, the effect of weak Faradic stimulation could be observed. The electrodes were applied to three different places: first, on the side of the chimney; second, at the base of the chimney, one wire on each side; third, at points on the flesh immediately back of the chimney at distances of from 3 mm. to 5 mm. from it. A weak stimulus when applied to the tip of the chimney caused a contraction of the oscular sphincter and a gradual

wave of contraction down the entire length of the chimney. This wave traveled down at the rate of about 1.5 mm. in four seconds. A strong stimulus applied to the chimney caused a complete relaxation, as though paralyzed, and from which it did not recover.

Stimulation at the Sides of the Chimney.

Choosing chimneys which were long and well extended the electrodes were applied at one side of the chimney, Fig. 3, *A*. When a weak stimulus was used, the response required about one minute before it appeared. A mild stimulus resulted in an immediate response. First, the chimney would bend toward the electrodes, the bending occurring at the points where the wires touched the chimney, Fig. 3, *B*. This bending always occurred within thirty seconds from the time the stimulus was started. At the same time, but continuing longer, the entire chimney would shrink slightly. This slow shrinking continued for from one to five minutes. If the wires were applied to the opposite side (Fig. 3, *C*) while the chimney was still bent as in (*B*), within one minute the end of the chimney would swing over in that direction, Fig. 3, *D*. Within thirty minutes the chimneys would straighten and expand to their former size and shape.

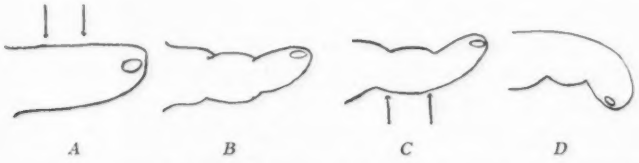

Fig. 3. The arrows indicate the points on the side of the chimney where the electrodes were applied.

Stimulation at Base of Osculum.

When a very weak stimulus was given for fifteen seconds with an electrode at each side of the base of the chimney, a very gradual longitudinal shrinking could be observed. The time required for the contraction to travel from the base to the tip was ten seconds, a rate of transmission of 0.17 mm. per second.

If the stimulus was a little stronger, an immediate contraction was followed by a slower general contraction, both lengthwise and in circumference, which would continue for about fifteen minutes, Fig. 4, *A* and *B*. The chimney would then begin to expand and within thirty-five minutes would be almost as large as before. On one occasion the sphincter at the base contracted more quickly than the others, resulting in the form of chimney shown in Fig. 4, *C*.

FIG. 4. The arrows indicate the points on each side of the base of the chimney where the electrodes were applied.

STIMULATION ON THE FLESH.

On several sponges the electrodes were applied immediately behind the osculum at distances of from 4 mm. to 2 mm. from it, and the flesh stimulated for periods varying from fifteen to sixty seconds. No effects could be seen on the chimneys.

There was, however, some indication of an excitation of the choanocytes. The currents were stronger and more rapid for from ten to fifteen minutes after the stimulation. Although this was taken as an indication of greater activity on the part of the flagellate cells, it is possible that the stimulation caused the ostia to dilate or open wider and in this way allow a larger volume of water to pass into the sponge body. It does not seem, however, that this would account for the increased rapidity with which the currents poured out of the oscula.

There was a noticeable difference in the responses of the oscula to Faradic stimulation depending on the strength of the electrical current used. From a weak stimulus,[1] a very gradual contraction was followed by a more rapid relaxation, while, if

[1] By a weak stimulus is meant one which could barely be detected when the electrodes were applied to the tongue, while by a mild stimulus is meant one that could be felt when the electrodes were applied to the lips.

the stimulus was mild, there would be an immediate contraction followed by a slower more general contraction. In this case the chimney would remain contracted for a few minutes and then expand much more slowly than it had contracted. In the second instance, all of the contractile cells probably were directly stimulated by the electrical current, while in the first case it is probable that only those where the electrodes were applied were directly stimulated, the gradual contraction being the result of transmission of the stimulus from one cell to the next.

In one instance, following a very weak stimulation for thirty seconds, it was possible to keep an accurate measurement of the time required for contraction and relaxation of the sphincter at the mouth of the osculum. No check was made on the general contraction or relaxation of the chimney. That the contraction was slower than the relaxation is shown by the following graph. The temperature of the water was about 27° C.

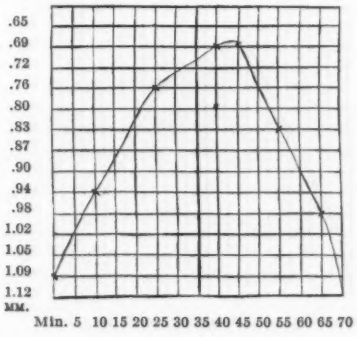

FIG. 5. Graph showing the rate of contraction and of relaxation of the sphincter at the mouth of an osculum following a weak Faradic stimulation. The abscissa represents the intervals of time in minutes and the ordinate the diameters of the opening in millimeters.

TRANSMISSION OF STIMULI.

It has already been pointed out that, apparently, very little transmission from one part of the body to another resulted from the application of the various types of stimuli used in these experiments. To determine if there was any transmission at all,

a sponge which had two large oscula at a distance of about 5 mm. apart was selected. A strong stimulus was applied with the electrodes at the base of one chimney. The result was an immediate jerk of the entire chimney followed by a gradual and complete relaxation of the entire chimney from which it did not recover. The other chimney was not affected.

There is, however, a noticeable transmission of stimuli in the chimney itself and the rate of this transmission is more rapid from the tip to the base than from the base to the tip. This is shown in the experiments with electrical stimulation and by rubbing the chimney with a needle. Rubbing the edge of the mouth of the osculum with a needle started a downward wave of contraction which could be seen to travel at the rate of about 0.25 mm. per second. Rubbing at the base produced no effect. Weak Faradic stimulation at the tip caused a downward wave of contraction with a rate of about 0.35 mm. per second, while stimulation at the base produced a slight wave of contraction which traveled toward the tip at the rate of about 0.17 mm. per second.

Conclusions.

Ephydatia fluviatilis is a fresh-water sponge which lives in either quiet or flowing water. Swift currents cause a shortening of the oscular chimney but do not cause a constriction of the osculum. Placing the sponge in a small quantity of quiet water results in a cessation of activities and death of the sponge in a short time, probably because of the metabolic products which accumulate in the water and poison the sponge.

The edge of the mouth of the osculum seems to be more sensitive to stimulation that the remainder of the chimney, as shown by the effect of rubbing it in various places with a needle.

Cutting the body of the sponge or sticking a needle into it seemed to have no effect except the local effect on the tissues which were injured. That there is no transmission of the effects to surrounding tissues, even for a distance of 2 mm. or 3 mm. from the injury, is shown by the continued functioning of the parts as indicated by the currents coming from the oscula and

by the fact that the chimney did not contract or in any way change its shape.

Subjecting the sponge to a low temperature does not have as disastrous an effect as increasing the temperature. Between 20° amd 30° C. seems to be approximately the temperature at which it thrives best. It is unable to live at a temperature of 40° C.

The oscular chimneys being continuous with the dermal membranes and themselves being contractile, one would expect to find the dermal membrane also contractile. It is capable of contraction to a slight extent under certain conditions. The chief contractile fibers, however, seem to be found only in the sphincters of the chimneys, which probably work against the general pressure of the water going out through the chimney.

Although in most cases, the responses of the fresh-water sponge were similar to the responses of *Stylotella*, there is one noticeable difference. This sponge responds to stimuli much more slowly, and the rate of transmission, where any at all is observable, is much slower.

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STUDIES IN MARINE ECOLOGY: I. THE DISTRIBUTION OF COMMON LITTORAL INVERTEBRATES OF THE WOODS HOLE REGION.

W. C. ALLEE,

MARINE BIOLOGICAL LABORATORY AND THE UNIVERSITY OF CHICAGO.

The distribution of animals within the Woods Hole region has been well studied by men interested in individual species and by those concerned with general faunistic problems. Some aspects of the ecology of the region are thoroughly set forth by Verrill and Smith in their "Report on the Invertebrate Animals of Vineyard Sound" made fifty years ago. This classic study remains the best account of the ecology of littoral species available.

The extensive "Biological Survey of the Waters of Woods Hole and Vicinity" [1] completed about ten years ago by Sumner, Osburn and Cole, while a mine of information concerning the animals of the region, was directly concerned with dredging operations and has little to say at first hand concerning the animals of the intertidal region or those found just below the tidal zone. Among other suggestions they recommend (p. 25) that the intertidal fauna should receive the same detailed attention that they have given to the bottom dwelling species.

In the absence of a report by the person best qualified to write on the subject, Mr. George M. Gray, the present series of papers has been prepared to make available information accumulated in nine consecutive summers' experience with the inshore invertebrates of the region.

The work has been done in connection with a teaching appointment in the course of Invertebrate Zoölogy of the Marine Biological Laboratory. It represents the collaboration of eighteen staff members and of about four hundred students. Many of the present collecting methods were installed in conjunction with Professor Caswell Grave, my predecessor in charge of the

[1] This report will be referred to hereafter as the *Biological Survey*.

course, but the records here used have been kept from the beginning by myself with the occasional help of other staff members.[1]

The records are based on the bi-weekly collecting trips of the Invertebrate Class and cover most thoroughly the period from about June 20 to August 15. These trips have been supplemented by expeditions made by instructors and by special trips for particular observations.

The organization of field work for eight years has been to divide the class into as many collecting teams as there were instructors. One person from each team was appointed recorder for the day and was supplied with a list of all the animals previously taken from the locality under consideration. The animals found were recorded according to habitats. The complete list for the year was made up from these combined records.[2]

[1] The following people have been at one time or another members of the instructing staff of the Invertebrate Course and have contributed to the data on which this series of papers is based. Without their coöperation this work could not have been done. Caswell Grave, Raymond Binford, E. J. Lund, George A. Baitsell, T. S. Painter, F. M. Root, W. J. Kostir, Robert H. Bowen, C. L. Parmenter, G. S. Dodds, Robert Chambers, Jr., Ann H. Morgan, W. J. Crozier, Donnell B. Young, J. P. Visscher, J. A. Dawson, Christianna Smith and E. A. Adolph.

I am indebted also to Mr. G. M. Gray for much valuable aid and friendly assistance; to Dr. Mary J. Rathbun for identification of the Brachyura; to Mr. Waldo L. Schmidt for similar service with the Anomura and Macrura; to Mr. Clarence R. Shoemaker for similar service with the amphipods and isopods; to Professor E. S. Morse for assistance with some of the molluscs and to Professor Raymond Osburn for assistance with the Bryozoa.

[2] The formal record of collecting experience has been recorded in abbreviated form on library cards which are deposited in the Library of the Marine Biological Laboratory. An annotated catalog of the distribution has been prepared as Study II. of this series and deposited with the library of the U. S. Fish Commission who have kindly agreed to furnish copies to the libraries of the Marine Biological Laboratory at Woods Hole; the Museum of Comparative Zoölogy at Cambridge, Scripps Institution at LaJolla; the United States National Museum at Washington and to the Harpswell Laboratory at Mount Desert Island, Maine.

The catalog shows the littoral invertebrates collected during the years 1915–1921 inclusive. Each locality in which an animal has been taken is recorded. The number of years which it has been found in a given locality is shown and an index figure of comparative abundance is also given. Where possible and desirable the location of particularly favorable collecting grounds is given with some exactness. This elaborated catalog forms the basis from which the facts presented here are drawn and together with the present report gives the background for the two following studies.

So far as possible, identification was done in the field. Doubtful specimens were referred from one instructor to another. Specimens new to the locality or difficult to identify were brought into the laboratory for further study. With the exception of the arthropods, few of the specimens have been referred to experts although we have gradually accumulated a type collection of animals found. The identification of animals has been made on the conservative basis that when doubt existed, the specimen was referred to the more common species. Wild identifications have been eliminated as far as possible, even to the extent of throwing out the entire reports of inexperienced instructors.

In spite of this care, mistaken identifications have probably been turned in and accepted. The list here given is substantially correct since the animals have either been reported by qualified collectors or placed on the list from demonstrated specimens. The imperfections lie largely in failing to distinguish closely related species and in possible errors in distribution records.

II.

The collections upon which this series of reports are based have been made largely in the littoral zone as defined by Edward Forbes; that is, between high water and a depth of two fathoms. This is not the littoral zone of modern zoölogists, but the term has been used with so great a variety of meanings that the extent of the study can be more easily and definitely located as being in the intertidal and ad- or sub-tidal regions.[1]

The intertidal zone is much restricted in the Woods Hole region on account of the slight rise and fall of the tides. The

[1] Murray and Hjort use the term "littoral zone" to include the region near the shore down to a depth of 30 or 40 meters: "almost as far as there are sea-weeds." It is frequently used as by Petersen to include the entire continental shelf. The botanists tend to be more exact. Kjellman limits the term to the region between extreme low and extreme high tide. Davis regards the littoral zone as extending from about mean low water to the highest point at which algæ can grow. Flattely and Walton ('22) follow Cotton ('12) and define the littoral region as extending from the level of highest marine vegetation, to low water at neap tide.

I prefer to use littoral in its original meaning of "pertaining to the shore"; intertidal or tidal zone adequately and exactly describes the region between the tide lines, and sub-tidal or adtidal are exact terms, if not the most correct etymologically, for the region below low tide. The question is discussed in the *Biological Survey*, p. 179.

tide tables of the U. S. Bureau of Commerce show a spring tide range of about five feet for this section of Buzzards Bay and only about two feet for Vineyard Sound stations.

Studies have been made in the following localities:[1]

Wharf Pilings.

Crane's Wharf Pilings.—This is a comparatively new wharf located near the public steamboat wharf in Great Harbor at Woods Hole. At the shore end the water at low tide comes close to the retaining wall and some collecting has been done annually in the crevices of the wall. The water at the outer end is over twelve feet deep. The number of species and of animals on these pilings has increased noticeably during the period of observation.

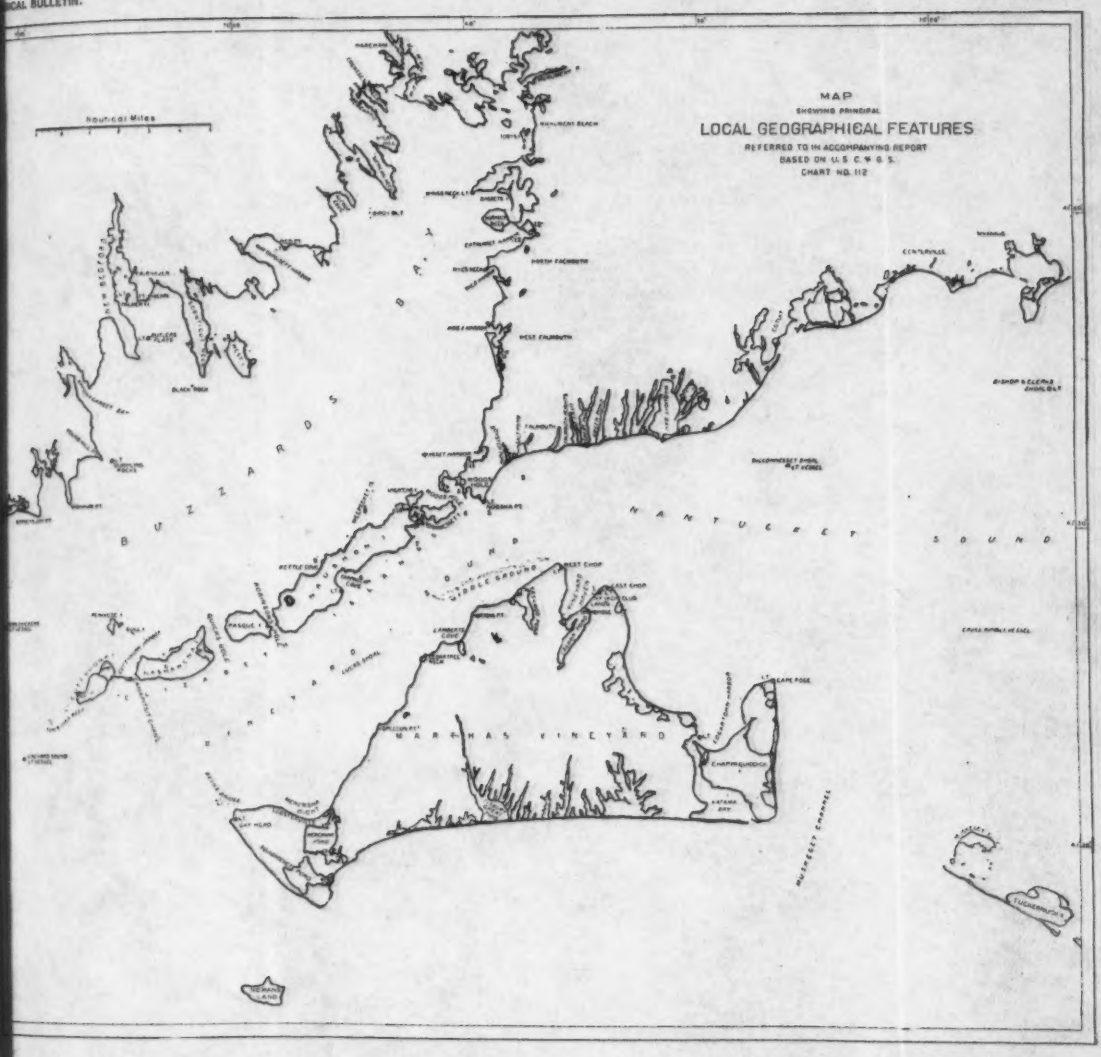
Vineyard Haven Wharf Pilings.—The old New York and Portland Wharf on the south side of Vineyard Haven is located well out toward the Sound. The water here comes up on a sandy beach which at low tide is bare for a considerable distance under the wharf. At the outer end the Government Chart shows 11 feet of water. In my experience the water is deeper. This is an old wharf with many pilings rotted off below water level. Some of the pilings are reproduced in the American Museum of Natural History in New York. Collections from both wharfs were made from boats by means of the usual scrape nets.

Marine Biological Laboratory Pier on Glass Slides.—For a number of years, glass slides have been placed under the M. B. L. pier in connection with other studies. In 1921 the slides were carefully examined by Dr. D. B. Young and myself after they had been suspended in water under the pier from July 1 to August 9 at a depth of about six feet. The M. B. L. supply float containing animals from all parts of the region was only a few feet away and accordingly more species were attached than might normally be found.

Rocks and Flats.

Hadley Harbor, Southwest and Southeast Gutters.—These naturally narrow rocky gutters have been further narrowed artificially

[1] Consult map.



Vineyard Haven Wharf.

Station 1, Vineyard Sound; near oyster beds.

Station 2, Vineyard Sound; *Chætopleura* grounds.

Station 3, Vineyard Sound; *Amaræcium pellucidum* grounds. Locality 1 of the dredging in Great Harbor is just within the harbor entrance.

Crane's Wharf. Localities 2 and 3 in Harbor dredging are nearby.

Lackey's Bay and Sound Gutters. The figure stands approximately on Blind Gutter Bar.

7. Station 4, Vineyard Sound; starfish hole.
8. Station 5, Vineyard Sound; sand dollar bed.
9. Kettle Cove.
10. Northwest Gutter Flats.
11. Gansett Bay.
12. North Falmouth Flats.
13. Squeteague Harbor in the North Falmouth Flats complex.

The map is taken from Bull. 31, U. S. Bureau of Fisheries.

so that they can be bridged. Strong tidal currents run through them the greater part of the day. They are relatively shallow, rock-walled channels, containing about six feet of water and are connected with open water by creeks which are also rock edged. Small patches of mud and sand occur frequently and the whole system of protected waterways supports many plants, *Ascophyllum*, *Fucus* and *Sargassum filipendula*.

Hadley Harbor Flats, Northwest Gutter.—Northwest Gutter separates Uncatena Island from Naushon. Before opening into Buzzards Bay it enlarges to form an approximately square expanse of shallow water about 250 yards along the south and west sides and about 400 yards in greatest diagonal. In most places the water is so shallow that it is difficult to push a boat along at low water. The sand bar over the channel of the gutter is fully bare at extreme low tide often to the extent of an acre or more.

The wide channel is kept scoured clean by the current, but behind the protecting sand and gravel spits, organic debris has accumulated to the depth of several feet and supports a rank plant growth composed chiefly of eel grass. At the Bay entrance there is the usual accumulation of rocks which extend off to a sand bottom some four feet below the lowest tide. The mud flats are bordered by rocks partially buried by mud.

Gansett.—Gansett is an offshoot of Quamquissett Harbor and has the same opening into Buzzards Bay. The main axis extends at an angle from the opening so that the back portion is usually protected from the direct drive of the waves. The opening is about 200 yards wide and the bay is approximately twice that length. At mid-mouth at mean low tide the water is 18 feet deep. The sides slope in rapidly near the shore so that there are only narrow strips of the different habitat zones. At the sides are the customary rocks and the outer corners are guarded by rock piles. At the head of the bay the shore is sand mixed first with gravel and lower with mud. Eel grass comes within two rods of the water's edge at low tide and thickly covers the bottom throughout its extent.

North Falmouth.—The collecting grounds here are scattered. They are located at the head of Cataumet Harbor and extend

over Squeteague Harbor which opens from the former by a winding narrow passage. Except for the dredged passage, most of the region can be waded at low tide. Much of the ground in Cataumet and almost all in Squeteague Harbor is left bare by the spring tides. The collecting is over a wide range of bottom: sand, mud, scattered rocks and gravel with and without eel grass and other sea-weeds. There is a somewhat sparse collection of rocks along the shore line.

Lackey's Bay.—Lackey's Bay belongs to the Hadley Harbor complex. It is located on the Vineyard Sound side between Naushon and Nonamasset. The part studied forms an expanded entrance to Middle Gutter which, by the construction of a causeway, has become Blind Gutter. The current is much diminished by the causeway and the inner part of the bay is deeply overlaid with muck. Eel grass is abundant. The region most studied is about 400 yards long by 200 yards wide and is separated from the Sound by a sand bar which is left bare at low tide.

Dredging.

The dredging has been largely in three localities in Vineyard Sound. These are the sand dollar bed (Map, No. 8) near the east side of the entrance to Tarpaulin Cove in about 20–30 feet of water. The bottom material brought up by the coarse dredge used is largely composed of shells. The starfish hole (Map, No. 7) is further east and still off Naushon, has about 90 feet of water. The *Chætopleura* grounds (Map, No. 3) off Nobska have about sixty feet of water. The bottom is decidedly pebbly. Some dredging has been done further east on or near the planted oyster bed (Map, No. 2) in Falmouth Harbor. The bottom here is sand and gravel in about 60 feet of water. In 1921 we dredged off the west entrance from Vineyard Sound to Great Harbor (Map, No. 4) in about 80 feet of water. This is over an *Amaræcium pellucidum* bed.

In 1920 we dredged in Great Harbor (Map, No. 5): at the east end of Nonamasset in 10–12 feet of water; in the Fish Commission Hole at a depth of 50 feet and at the West end of the passage in Woods Hole in about 20 feet of water.

The dredging work has been largely incidental and the results are given chiefly as a means of comparing the more extensive

results obtained by the dredgings of the *Biological Survey* with our main work further inshore.

A Habitat Check List of the Common Invertebrate Animals of the Woods Hole Littoral with Distribution Records for 1920 and 1921.

The appended list of animals is based on all the collecting done since 1912. The distribution records are based on the reports from operations in 1920 and 1921. The statistics given are from team records. Thus in these two years, two teams collected *Chalina* from the mud, eight teams have recorded it from rocks, ten from wharf pilings and nine from dredging. The figures given show no indication of the number of specimens taken other than that suggested by the fact that the more animals present, the greater the probability that all teams would find them. Anyone interested in the abundance of these animals in particular localities is referred to the second study in the present series.

The tabulation is from the reports of 30 collecting teams operating on wharf pilings; 52 from sand, mud, gravel and eel grass; 56 teams from rocks and 42 from dredging. The records of plankton have been kept in a different way and the presence of recognized animals in late July or early August is merely checked.

The classification of habitats in the field has sometimes been left to the judgment of the student recorder and it is entirely probable that some of the 52 recorders thought a given habitat was best recorded as "sand" while others regarded animals from a similar place as "mud" dwelling. All gradations between the two exist and the conditions under which the collecting was done do not permit a more refined grading. The error arising from this source is somewhat compensated by the fact that no dragnet collections were made as it was desired to find where individual animals live as well as to collect different species.

Unidentified animals have not been included in the habitat list unless the genus, at least, could be determined with some assurance. All the records are for living animals since for ecological purposes the recording of dead shells can only be worthless and confusing in a region where tidal currents run strongly and where the shore birds distribute shells even over the land.

The nomenclature follows Pratt wherever the species are listed in his *Manual*. Other species have the name given them in the catalog of the *Biological Survey*. No attempt has been made to give synonyms since these can usually be found in the *Survey* catalog.

The arrangement of species within the major divisions is alphabetical. While this does violence to all principles of taxonomy, the taxonomic sense is not strongly developed at present, and this method renders the material more easily available to the average zoölogist than would be the case if a strictly taxonomic system were followed.

The records given in the habitat list are necessarily abbreviated. Thus, *Hydractinia* is recorded as taken from "sand" when the whole record should read: "on shells inhabited by hermit crabs taken on sandy bottom"; or other animals, as *Sagartia luciæ*, recorded from "mud" which does not mean that the anemone was growing on the mud but that it was found attached to a bit of board or rock surrounded by typical mud conditions.

The classification headed "eel grass" includes records of animals living free among the eel grass, as *Pecten;* attached to eel grass, as *Pennaria;* crawling over it, as *Ophioderma;* on the substratum at its base, as *Microcione;* or burrowing in the substratum at its roots, as *Cumingia*. In addition the lumping is still greater for one must remember that eel grass begins to grow on fairly pure sand and extends back to the pure muck of the flats.

A number of animals are recorded under "rocks and rock-weeds" which were taken only from the substratum under or among the rocks. Whenever all the records are for an animal so found, the entry has been appropriately labeled.

Under "range" is listed the information at hand showing the distribution of the animal along the Atlantic Coast. The abbreviations are: N., north ranging; S., south ranging; M., approximately mid-range; L., local; C., cosmopolitan. Whenever an animal is known to extend twice as far north of Woods Hole as south, it is listed as north ranging and *vice versa*. (Cf. Hoyle, 1889). Some relations between the local and geographical distribution will be discussed in a later paper.

Species.	Range.	Unclassified.	Mud.	Sand.	Gravel.	Eel Grass.	Rocks and Rockweed.	Pilings.	Dredging.	Plankton.
			No. Teams Reporting.							
			52	52	52	52	56	30	42	
PROTOZOA.										
List not given.										
PORIFERA.										
Chalina arbuscula	S.		2				8	10	9	
Cliona celata (?)	S.			2		1	33	25	16	
Grantia ciliata	N.			1			11	30	13	
Leucosolenia botryoides	N.						13	24	1	
Microciona prolifera	S.		1			4	43	30		
Tethya gravida	L.		1						1	
CŒLENTERATA.										
Hydrozoa										
Abietinaria abietina	N.								12	
Bougainvillia carolinensis	S.					1	2	1		✓ sp.?
Campanularia calceolifera								1		
Campanularia sp.?							1	5		
Clava leptostyla	N.						1			
Clytia grayi	L.						2			
Clytia bicophora	N.					1	1	1		✓ sp.?
Clytia cylindrica						1				
Eucheilota sp.? *medusæ*										✓
Eudendrium album						2	4	6		
Endendrium ramosum	M.						7	22	7	
Eudendrium tenue								1		
Gemmaria gemmosa	S.			1			1	1		✓
Gonionemus murbachii	L.									
Hydractinia echinata	N.		9	18	3	5	12		11	
Obelia bicuspidata	S.					1		1		
Obelia commissuralis	S.		1			12	8	11	2	✓ sp.?
Obelia geniculata	N.					5	6	7		
Pennaria tiarella	S.					14	4	10	4	✓
Phialidium sp.? *medusæ*										✓
Podocoryne carnea	N.			2		1				
Schizotricha tenella	S.						1	11	2	
Sertularia pumila	N.					11	30	12	10	✓
Stylactis hooperi	S.									✓
Tubularia crocea	S.							30	7	✓
Scyphozoa.										
Aurelia flavidula	M.									
Cyanea capillata arctica	N.									
Dactylometra quinquecirrha	S.									
Haliclystus auricula	N.									

Species.	Range.	Unclassified.	Mud.	Sand.	Gravel.	Eel Grass.	Rocks and Rockweed.	Pilings.	Dredging.	Plankton.
			No. Teams Reporting.							
			52	52	52	52	56	30	42	
Anthozoa.										
Astrangia danæ	S.						11		7	
Alcyonium carneum	N.									
Edwardsia elegans	N.		1	5		1				
Eloactis producta	S.			3						
Metridium dianthus	N.						29	24		
Sagartia leucolena	S.		3	3		2	37	11	4	
Sargartia luciæ	S.		2	2		6	38	6	2	
Sagartia modesta	S.					4	12 under			
Platyhelminthes.										
Turbellaria.										
Bdelloura candida	S.	3	5	2		7				
Bdelloura propinqua		1								
Polychœrus caudatus	N.		1	3		5	2	1	1	
Procerodes wheatlandi	N.						1			
Stylochus ellipticus	N.		3	2	1	1	6	3		
Stylochus zebra	L.						1		1	
Syncœlidium pellucidum						2				
Nemertini.										
Amphiporus ochraceus	S.								5	
Cerebratulus lacteus	S.		7	11		2	2			✓
Cephalothrix linearis	N.						under 1			
Lineus sp.?			2	6		5	under 1		2	
Lineus bicolor	S.		1	1			1	2		
Micrura leidyi	M.		6	15		8	3			
Tetrastemma vermiculum	S.						under	5	3	
Nemathelminthes.										
Pontonema marinum	S.		1	1		5	6	19	6	
Echinoderma.										
Asteroidea.										
Asterias forbesi	S.		1	8		4	28	14	29	✓
Asterias vulgaris	N.							1	4	
Henricia sanguinolenta	N.						6	4	11	

Species.	Range.	Unclassified.	Mud.	Sand.	Gravel.	Eel Grass.	Rocks and Rockweed.	Pilings.	Dredging.	Plankton.
			No. Teams Reporting.							
			52	52	52	52	56	30	42	
Ophiuroidea.										
Amphipholus squamata	N.						2		12	
Ophioderma brevispina	S.					5				
Echinoidea.										
Arbacia punctulata	S.			6			18	2	17	✓
Echinarachnius parma	N.						1		12	
Strongylocentrotus dræbachiensis	N.									
Holothuroidea.										
Leptosynapta inhærens	S.		11	24		10				
Thyone briareus	S.		25			6			2	
ANNELIDA.										
Archiannelida.										
Dinophilus sp.?										✓
Polychæta										
Ampharete setosa	S.									
Amphitrite attenuata							1	9		
Amphitrite brunnea	N.							1		
Amphitrite ornata	S.		19	8		3	3		2	
Arabella opalina	S.		8	17	2	5	under 1	1	7	
Arenicola cristata	S.		3	6			under 1			
Autolytus cornutus	N.					1	under	9		
Autolytus varians	S.							1		
Chætopterus pergamentaceus	S.					1				
Cirratulus grandis	S.		11	6	1		5		1	
Cirratulus tenuis							under		1	
Capitella sp.?										
Clymenella torquata	M.		17	25		6	6			
Diopatra cuprea	S.		6	14		2	under 1		2	
Drilonereis longa			1	7	1		under			
Enoplobranchus sanguineus	M.		3							
Glycera americana or	S.	}	20	53	1	12	1			
dibranchiata	M.	}					under			
Harmothæ imbricata	N.		2	5	1	3	26	27	11	

Species.	Range.	Unclassified.	Mud.	Sand.	Gravel.	Eel Grass.	Rocks and Rockweed.	Pilings.	Dredging.	Plankton.
			No. Teams Reporting.							
			52	52	52	52	56	30	42	
Polychæta Con't.										
Hydroides hexagonus	S.	5	2	3	1	3	39	17	22	
Laonice viridis	S.	3	3	1						
Lepidametria commensalis	S.						1 among			
Lepidonotus squamatus	N.	1	3	5	2	2	37	29	22	
Lepræa rubra	S.				1		4 under			
Lumbrinereis hebes										
Lumbrinereis tenuis	S.		7	19	2	1	3 under		14	
Maldane urceolata	S.		3	9		2				
Marphysa leidyi			1						3	
Nereis limbata	S.		12	9	2	3	8	3		
Nereis pelagica	N.		4	6			9	29	22	sp.?
Nereis virens	N.		17	20		9	3 under	1		
Nicolea simplex			1	3		2	7	13	7	
Pectinaria gouldi	S.		9	18	1	5				
Potamilla sp.?									1	
Pholoë sp.?										
Phyllodoce catenula	N.	1	3	6		3	5	17	13	
Pista palmata	S.		3	1		1				
Platynereis megalops	S.					1		1		
Podarka obscura	S.		2			4	4	7	6	
Polycirrus eximeus	S.		10	14		4	15	6	12	
Polydora sp.								2		
Sabella microphthalmia	S.		1	1	2		3	7	1	
Sabellaria vulgaris	S.	1	2	2		5	6	2	4	
Scoloplos acutus			12	1		6				
Scoloplos fragilis	S.		8	19		9	1 under			
Scoloplos robustus	S.		8	10		3	1 under			
Spio (setosa?)				1						
Spirorbis spirorbis	N.		3	4	2	11	26	19		
Spirorbis tubæformis	S.						1		1	
Sthenelais leidyi	S.		6	5		4	3	4	6	
Terebellides stræmi	N.									
Thelepus cincinnatus	N.						1	3		
Trophonia affinis	S.		4	1					6	
Chætognatha and Sipunculoidea										
Phascolosoma gouldii	M.		13	22		3	2 under			
Sagitta sp.?										✓

Species.	Range.	Unclassified.	Mud.	Sand.	Gravel.	Eel Grass.	Rocks and Rockweed.	Pilings.	Dredging.	Plankton.
			No. Teams Reporting.							
			52	52	52	52	56	30	42	
BRYOZOA.										
Ælea anguina	N.					5	10	12		
Alcyonidium sp.?							3			
Bicellaria ciliata	M.							3	3	
Bowerbankia gracilis	N.						3	1		
Bugula cucullifera	N.							1		
Bugula flabellata	N.						1	1		
Bugula turrita	S.		1	2		17	17	31	14	✓
Cribrillina punctata	N.								1	
Crisia eburnea	N.		1	1		4	21	18	12	
Flustrella hispida	N.					1	22			
Hippothoa divaricata	C.							1		
Hippothoa hyalina	C.							1		
Lepralia pallasiana	N.									
Lepralia pertusa	S.	1		1		10	14	6	6	
Lepralia serrata	M.							1		
Lichenopora verrucaria	N.							2		
Membranipora monostachys								1		
Membranipora pilosa	N.			1	1	6	21	13	5	
Membranipora tenuis									6	
Microporella ciliata	C.						1	2	3	
Schizoporella biaperta									5	
Schizoporella unicornis	S.		2	3	1	8	32	25	11	
Smittia trispinosa nitida	M.					1	3		15	
ARTHROPODA.										
Phyllopoda.										
Evadne nordmanni	M.									✓
Podon leuckarti	M.									✓
Cirripedia.										
Balanus balanoides	M.		5	4	3	2	48		2	✓
Balanus eburneus	S.		1	3	1	6	18	19	17	✓
Lepas anatifera	C.	1					1			✓
Arthrostraca.										
Amphithæ rubricata	N.		1	1			6	6	8	
Autonœ (Lembos) smithi	L.			1			5	5	9	
Æginella longicornis	N.						1	4		
**Caprella geometrica*	S.			1		6	13	26	1	✓
Chiridotea cæca	S.		2	2		2	2			
Corophium cylindricum								2		
Cyathura carinata	N.			1		1				
Edotea triloba	M.	1	3			2	2			

* In part *Æginella*

Species.	Range.	Unclassified.	Mud.	Sand.	Gravel.	Eel Grass.	Rocks and Rockweed.	Pilings.	Dredging.	Plankton.
			No. Teams Reporting.							
			52	52	52	52	56	30	42	
Arthrostraca Con't.										
Erichsonella filiformis	N.			1		2	2	2	4	
Gammarus (Sev. sp.)		2	8	11	3	15	10	28	18	✓
Haustorius arenarius	S.			2						
Idothea baltica	M.		5	4		20	9	15		✓
Idothea metallica	S.		3		2	5				✓
Idothea phosphorea	N.						1			
Jæra marina	N.	2		2	1	4	3			
Ligyda oceanica	N.						3			
Orchestia agilis	S.	10	1	33			8	2		
Sphæroma quadridentatum	S.						5			
Talorchestia longicornis	S.		2	14	6	1				
Tanais cavolinii	N.							1		
Unicola irrorata	N.								1	
Thoracostraca.										
Callianassa stimpsoni	S.		1				1 among			
Callinectes sapidus	S.		1	1						
Cancer borealis	N.					3				
Cancer irroratus	M.		5	7	2	4	6	2 near	2	
Crangon vulgaris (*Crago septemspinosus*)	M.		3	4	4	16	3		1	
Carcinides mænas	S.		16	4	4	4	8		5	
Heterocrypta granulata	S.								1	
Heteromysis formosa	N.	1	3	3		1	1		12	✓
Hippa (*Emerita*) *talpoida*	S.									
Hyas coarctatus	N.									
Libinia dubia	S.		14	8	1	10	3	20	16	
Libinia emarginata	S.		14	4	1	7	1	10	9	
Lysiosquilla armata										
Mysis stenolepis	M.									
Michtheimysis mixta										
Ovalipes ocellatus	S.		1	3		3	2		1	
Pagurus acadianus	M.			1					2	
Pagurus longicarpus	S.	3	19	16	5	10	7	2	22	
Pagurus pollicaris	S.	1	4	10	4	7	1	1	10	
Palæmonetes vulgaris	S.		9	7		16	8			
Panopeus sayi or *Neopanope texana sayi*	S.	2	17	12	3	12	7	27	16	
Pelia mutica	S.			1						
Pinnixa chætopterana			2			2	1 among			
Pinnixa sayana (*cylindrica*)										
Pinnotheres maculatus	S.		3					6		
Portunus sayi	S.									
Squilla empusa	S.									

Species.	Range.	Unclassified.	Mud.	Sand.	Gravel.	Eel Grass.	Rocks and Rockweed.	Pilings.	Dredging.	Plankton.
			No. Teams Reporting.							
			52	52	52	52	56	30	42	
Thoracostraca Con't.										
Uca pugilator	S.		21	4	3	2	8			
Uca pugnax	S.			2						
Virbius (Hippolyte) zostericola	S.	1	4	2		25	1			
Arachnoidea.										
Anoplodactylus lentus	N.	2					4	10	2	
Pallene empusa	S.					2	1	5		?
Limulus polyphemus	S.	1	12	10	2	6	1			
Tanystylum orbiculare	S.							10	1	
MOLLUSCA. *Amphineura.*										
Chætopleura apiculata	S.	3					19		16	
Gasteropoda.										
Acmæa testudinalis	N.	2	1	2		1	26	1	3	
Bittium alternatum	M.	1	6	6	2	20	17	11	8	
Busycon canaliculatum	S.			7	1		2			
Busycon carica	S.			1	1		1	1		
Cæcum pulchellum							2			
Cerithiopsis emersonii	S.	1				2	3		6	
Cerithiopsis greenii	S.					2	1 sp.?		2	
Cerithiopsis terebralis									2	
Columbella avara	S.	1	3	5	1	9	13	14	19	
Columbella lunata	S.	1	3	7		12	20	30	21	
Coryphella gymnota	N.	1			1	1	2	15		
Crepidula convexa	S.	4	12	10	1	6	20	4	7	
Crepidula fornicata	S.	2	6	15	1	2	21	12	9	
Crepidula hlana	S.	3	7	16	2	2	15	14	15	
Doris bifida	N.							1	1	
Elysia chlorotica	M.					4	1			
Eupleura caudata	S.		1	3	1		4		5	
Lacuna vincta	S.		4	4	2	14	13	21	9	
Littorina irrorata	S.						2			
Littorina litorea	N.	1	15	8	6	18	29	27		
Littorina palliata	N.		4	5	3	11	24	7	1	
Littorina rudis	N.		8	4	2	12	32	6	1	
Melampus lineatus	S.		14	4	2					
Nassa obsoleta	S.		35	14		3	9			
Nassa trivittata	S.		13	16	1	3	8	11	3	
Nassa vibex	S.		1							
Natica duplicata	S.		2	6	1	3	1 among			
Natica heros	M.		2	1	1		2			

Species.	Range.	Unclassified.	Mud.	Sand.	Gravel.	Eel Grass.	Rocks and Rockweed.	Pilings.	Dredging.	Plankton.
		No. Teams Reporting.								
			52	52	52	52	56	30	42	
Gasteropoda Con't.										
Natica immaculata	N.								2	
Natica pusilla	S.		1			1				
Odostomia sp.?		1	2	1		7	24	2		
Rissoa minuta	N.		3							
Rissoa sp.?										
Scalaria sp.?			1	1						
Purpura lapillus	N.			1			10			
Urosalpinx cinerews	S.	4	5	8	1	5	31	22	5	
Pelecypoda.										
Anomia aculeata	N.					1	12	4	5	
Anomia ephippium	S.		2			1	22	10	7	
Arca pexata	S.			1			5	1	7	
Arca ponderosa	S.									
Arca transversa	S.		1	1			1		4	
Astarte castanea	M.								3	
Astarte undata	N.								5	
Cardium pinnulatum	N.								5	
Clidiophora trilineata	S.								3	
Corbula contracta	S.			2						
Cumingia tellinoides	S.		10	11		6	1			✓
Ensis directus	M.		9	9		2	5			
							among			
Gastranella tumida	S.							1		
Gemma gemma	N.			3			1			
Lævicardium mortoni	S.		11	5	2	4	1			
							among			
Lyonsia hyalina	S.	1		2						
Macoma tenta	S.		2	2		1			1	
Mactra lateralis	S.			1						
Mactra solidissima	N.			3			2		2	
Modiolus demissus	S.		22	15	3	6	8			
Modiolus modiolus	N.		10	7		4	6	3	3	
Mya arenaria	N.		23	21	3	6	5			
							among			
Mytilus edulis	N.		11	11	5	4	24	28	7	
Nucula delphinodonta	N.								1	
Nucula proxima	M.		3	3	1	4	4		3	
							among			
Ostrea virginica	S.		10	5			9			
Pecten irradians	S.		9	9		11	4		4	
Petricola pholadiformis	S.		3	2	3		1			
Saxicava arctica	N.						2			
Solemya velum	M.		10	20		4	4			
							among			
Tellina tenera	S.		5	16	3	4	1			
Teredo navalis	N.	19		2	1	1	1	7		

Species.	Range.	Unclassified.	Mud.	Sand.	Gravel.	Eel Grass.	Rocks and Rockweed.	Pilings.	Derdging.	Plankton.
			No. Teams Reporting.							
			52	52	52	52	56	30	42	
Pelecypoda Con't.										
Venus mercenaria	S.	1	19	14	2	1	4 among		2	
Yoldia limatula	N.									
Zirphæa crispata	N.		1							
Cephalopoda.										
Loligo pealii	S.			3						✓
CHORDATA.										
Appendicularia longicauda	M.									✓
Amarœcium constellatum	N.	3			2	3	19	29	14	
Amarœcium pellucidum	S.								12	
Amarœcium stellatum	S.									
Botryllus schlosseri	N.	1				13	7	5		✓
Didemnum lutarium	N.	2				6	13		30	
Dolichoglossus kowalevskyi	S.		12	14	6	2	3 under			
Molgula manhattensis	S.	2	3		1	6	8	24	1	
Molgula papillosa	N.							1		
Molgula arenata	S.							1		
Perophora viridis	S.	2		1		5	16	28	4	
Styela partita	S.		1	1		6	17	29	9	

IV.

The entire mass of data available was analyzed in 1917 and again in 1920 in an attempt to discover the relationships existing between animal associations from the different types of environments. The first type of analysis was planned to discover the number of species common to different combinations of these habitats and conversely to find the number of animals peculiar to each type of habitat. The analysis was not repeated after the 1921 records were available because it was not considered that these records would materially change conclusions already arrived at by the preceding work.

The analysis of the records from 1915–1920 inclusive follows:

Habitats.	Number of Species.
Flats, rocks, pilings and dredgings	54
Flats, rocks and pilings	16
Rocks, pilings and dredging	10
Flats, pilings and dredging	3
Flats, rocks and dredging	9
Flats and under or on rocks	26
Wharfs and rocks	8
Flats and rocks	11
Dredging and rocks	5
Flats and pilings	2
Pilings and dredging	2
Flats and dredging	9
Flats, under rocks and dredging	4
On or under rocks	10
Pilings	7
Dredging	13
Only under rocks	1
Flats	41

The analysis shows that at the close of the 1920 season 54 species had been recorded from all four types of habitats studied while only 16 species were limited to and found in all of the three habitats excluding dredging. The flats have the greatest number of peculiar species, with 41, and but few forms are limited to any one of the other habitats.

The same material analyzed in another fashion is shown in Table 1. Here the attempt is to show the total numbers of

TABLE I.

	Wharfs.	Flats.	Rocks.		Dredging.
Wharfs	110 100%	83 74%	80 73%	76 69%	(26 only in Harbor)
Flats	83 44%	187 100%	80[1] 43%	87 46%	(28 only in Harbor)
Rocks	80 72%	80 72%	111 100%	81 73%	(22 only in Harbor)
Dredging	76 64%	87 73%	81 69%	119 100%	(32 only in Harbor)

animals found in each of the four major divisions of the littoral zones of the region in comparison with each of the other divisions.

[1] 30 more dug occasionally among or under rocks.

Again the analysis follows the records through the season of 1920. The comparison is based on total distribution records as was the last. That is, for the purposes of this table the finding of a given animal once in a given type of habitat is as significant as though it were abundant there. There were 242 species in the catalogue when this study was made.

It is immediately apparent that this type of analysis serves only to call attention to the larger number of animals taken from the flats and beyond indicating the higher specificity of the flats, shows no evidence of relationships that may exist between different habitats. The species taken from any given type of habitat are found to be approximately equally distributed in the other habitats of the region. Such experience has led casual observers to conclude that relationships between different animal associations can not be analyzed.

In order to make such an analysis, the records were studied from another angle. Species approximately equally distributed through the different associations and those reported for one season only were eliminated. Then the remaining species were listed according to the habitat in which they are most abundant. When a species was found equally abundant in two habitats it was listed from both. The association in which the animal was next most abundant was also estimated. Unfortunately these records are based on "experience" cards that seldom give specific figures and on the number of collecting teams that have reported the species from the different associations, as in the data given in the check list, rather than on strictly quantitative grounds and while substantially correct, they lack the finality that statistical treatment would give.

This type of analysis gives a real basis for comparisons of the relationship between the different associations. It is of interest that the relationships shown by Table II. are practically the same as given by an analysis of the entire catalog in 1917. In other words, the early collecting gave the typical forms characteristic of the environment while much of the later work has yielded in addition to these characteristic species, a number of accidental or incidental records.

TABLE II.

RELATION OF DIFFERENT HABITATS BASED ON DISTRIBUTION OF CHARACTERISTIC ANIMALS.

Name of Association.	No. Species.	Peculiar Species.		Species Found Next Most Abundant in:									
				Pilings.		Rocks.		Flats.		Dredging.		Under or Among Rocks.	
		No.	%.	No.	%.	No.	%.	No.	%.	No.	%.	No.	%.
Wharf pilings.....	32	2	6	—	—	24.5[1]	**77**	2.5	8	2.0	6	0	0
Rocks and rockweed	32	3	9	14.5	**45**	—	—	13	41	1.5	5	0	0
Dredging.....	20	3	15	3	15	9	**45**	3	15	—	—	0	0
Flats.....	40	22	25	2.5	3	15	17	—	—	17.5	20	30	**33**

The association of the wharf pilings is found to be closely related with that of the rocks; 77 per cent. of the animals common in the former are next most abundant in the latter. This fits with one's general impression that the two sets of animals are much the same but with the emphasis placed on different species so far as numbers are concerned.

The animals common on the rocks are next most abundant on the pilings but almost as many are nearly as abundant on the flats. The latter is to be expected from the fact that the rocks extend up from the flats, often forming a belt only a few feet wide in the intertidal portion of the flats, and that single rocks frequently occur surrounded by typical mud or sand flats, and from the further consideration that the eel grass offers almost as good a place of attachment for many animals as do the rocks.

The animals taken commonly in dredging are more closely related to the rock association than to the others. This is because our dredging operations have been done on clean hard bottom where the water conditions are similar to those found among rocks. Dredging in mud as in Buzzards Bay would give different results and transition bottom associations are indicated from the dredging work in Great Harbor.

Again the marked independence of the flats as a special habitat is shown by the larger number of animals frequently taken there

[1] Where animals are approximately equally distributed between two different associations they are summarized by fractional representation.

in some abunbance and by the larger number of peculiar species. They are not closely related to other habitats if one excepts the conditions under the rocks which scarcely form a different association. Its main difference is that burrowing and exposure are more limited than on the open flats. The animals common on the flats are particularly absent from wharf pilings showing, as would be expected, that these habitats have little in common.

If quantitative data were available, the distinctions here found would doubtless stand forth more plainly. The fact that a beginning can be made in indicating relationships by the methods used when no relationships appeared from an analysis of bare check lists emphasizes the need of quantitative studies in animal ecology.

This need was first recognized by Forbes (1907) who devised a mathematical formula for determining the existence of an association. In 1911, as the result of studying seasonal succession in ponds, I concluded that qualitative work gives insufficient basis for exact conclusions. Shelford in 1915 repeats the formula of Forbes, and Michael (16, 21) has done more than anyone else in America in showing the fundamental need of quantitative investigations in ecology and in developing formulæ to enable one to study associations on a quantitative basis.

The work here presented is of course only quasi-quantitative in character but the greater clearness obtained indicates that much of the muddle of animal ecology may be cleared by the further development and the application of quantitative methods in field researches. The problem of the ecologist studying littoral distribution is not so hard as that of the plankton student where as Michael says (1921): "Granting the equivalent of the oak tree or pine tree association, the marine ecologist finds difficulty not only in describing it but even in finding it. Since he cannot directly witness such an association, he is compelled to rely on indirect evidence furnished by tow-net or similar apparatus. In other words his only recourse is to measured magnitudes and application of mathematical logic thereto." For the exact determination of such relations the methods here used are almost as gross as are the ordinary qualitative observations in trying to solve the relationships existing between littoral associations.

It is true that analysis of the results of preliminary collecting showed the same relations as the quasi-quantitative analysis of more complete records in the Woods Hole region. Unfortunately one cannot be sure that the animals found in such preliminary work are really the typical animals since they may obviously contain many incidental forms. In other words in a random sample one is more apt to collect animals typical of the habitat than incidental forms but he can never be sure of this without further work.

V. The Effect of Continued Collecting on Distribution Records.

In 1917 when the collecting records were first studied seriously there were 181 species in the catalog. In 1920 when a similar study was made, the catalog listed 242 species. In the interim the Sound Gutters, Lackey's Bay and Great Harbor had been added to the localities visited.

In 1917, 11 species were recorded only from the wharf pilings. The later lists show 7 species so limited but this includes only one (*Tetrastemma*) of the previous list. In 1917 two species were recorded only from rocks or rockweeds, while on the later list there were 10 such species including only *Clava leptostyla* from the preceding list. In the first comparison there were 10 animals recorded only from dredging; in the later one, 13, which includes four of those on the preceding list: *Arca ponderosa*, *Strongylocentrotus droebachiensis*, *Heterocrypta granulata*, and *Amaroecium stellatum*.

On the 1917 list, 71 species were recorded from the flats only. After four more years' work this had shrunk to 41 providing animals found in the sand under and among rocks are excluded. Of these only 17 appear on both lists. They are: *Edwardsia elegans*, *Eloactis producta*, *Bdelloura candida*, *Syncoelidium pellucidium*, *Ophioderma brevispina*, *Chaetopterus pergamentaceous*, *Platynereis megalops*, *Scoloplos acutus*, *S. robustus*, *Spio* (*setosa?*), *Callianassa stimpsoni*, *Mysis stenolepis*, *Squilla empusa*, *Melampus lineatus*, *Clidiophora trilineata*, *Pecten irradians*, and *Tellina tenera*.

On the earlier lists, fifteen species were recorded from some place in each of the four main types of habitats: wharf pilings, rocks, flats, and dredging. In 1920 this list had increased to 54. These results mean, as has already been suggested, that as collecting has proceeded, animals have been picked up in habitats in which they are not abundant. The scarcity of many of these is shown by the number of single specimen records on the lists. There is little doubt but that if the present type of collecting were continued long enough, there would finally be stray records of many of the animals found in the region from each type of habitat. Even dredging, which we have usually carried on in deep water in Vineyard Sound, yields a different type of animals and becomes more closely related to other habitats as a result of dredging records taken in Great Harbor. In some one or more of their dredging operations, the *Biological Survey* found most of the animals we have taken from inshore digging. This result might be expected from the fact that some of their dredgings are recorded from less than 10 feet of water. If made at high tide, these would be almost as close inshore as our deepest collecting on wading and digging expeditions when we often collect out to four feet of water at low spring tides.

In other words, in such a small region as we are now considering, provided with strong tidal currents which aid in distribution, the animals tend to become widely distributed and occasional specimens will be found that can tolerate for a time conditions that are essentially unfavorable. Under these conditions the mere record of the presence of a species in a given habitat means very little unless there is due consideration of its abundance and duration in that locality. One is thus driven again to the conclusion of the last section, that quantitative work is necessary before final judgment can be passed in the matter of the constitution of animal associations.

We have found no evidence that the long continued collecting over the same grounds by the Invertebrate Class, nor the commercial collecting of the Supply Department of the M. B. L. has affected the number of animals present within the past nine years sufficiently for the effect to be noticeable by the collecting methods we have used. With growing experience in collecting

each year we have broken previous records with monotonous regularity, for numbers of species from most of the localities we visit. This could not have continued so long had the animals been becoming less abundant.

The number of animals present in a given locality must depend more on the availability of suitable breeding places and abundance of food than upon such disturbing influences as summer collecting, particularly when the collecting does not reach all the breeding habitats of a region and there is adequate means of distributing young stages. This conclusion is emphasized by the rapid recovery in numbers of *Arbacia* after their almost complete disappearance following the winter of 1917–18 (Allee, '19) and in the face of their destruction by the thousands in the research work carried on in the Woods Hole laboratories.

VI. Summary.

1. Analysis of distribution records in the four major types of habitats of the Woods Hole littoral, viz., wharf pilings, rocks and rockweeds, flats, and the sea bottom in deeper water show that mere records of species present in the different habitats fail to indicate any relationship between the different types of associations.

2. By eliminating species known to be approximately equally distributed throughout and records for one year, only, and classifying the remaining species in terms of places where they are most abundant and next most abundant one finds:

(*a*) The association of the wharf pilings is closely related to that of the rocks.

(*b*) Species taken in dredging on clean hard bottom are found in next abundance on the rocks.

(*c*) The associations of the flats are highly independent of the others in the region but continue in the mud and sand under and around rocks.

(*d*) That some degree of quantitative work is necessary in order to determine the relationships of animal associations.

3. Preliminary collecting in a region tends to give the obvious forms and gives similar results in analysis to the type of quasi-qualitative work described in this report.

4. The number of animals present in the Woods Hole region has not been noticeably affected by the intensive collecting carried on there during the nine years covered by these studies.

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FOOD AND PARTHENOGENETIC REPRODUCTION AS RELATED TO THE CONSTITUTIONAL VIGOR OF *HYDATINA SENTA*.[1]

W. V. LAMBERT, W. S. RICE, AND H. C. A. WALKER.

There has been a general belief for many years that cross-fertilization is beneficial to a race. Darwin studied the effects of inbreeding and crossbreeding in many plants and came to the general conclusion that while inbreeding has a deleterious effect, crossbreeding has a beneficial effect upon the races involved.

In experiments with infusoria Calkins has found that if conjugation is prevented the race gradually weakened and finally died. He observed, however, that by the artificial stimuli of various chemicals in the culture water he was able to prolong the life of one race of *Paramecium caudautam* to the 742d generation.

Investigations in regard to the effects of parthenogenetic reproduction have been carried on by Shull and Whitney with the rotifer, *Hydatina senta*. Both of these investigators have found that continued parthenogenesis in pedigree races of rotifers has resulted in a gradual weakening and loss of vigor, and in some experiments death. In Whitney's experiments one race of rotifers died out from general exhaustion in the 384th generation and another in the 546th generation. A third race, discontinued in the 443d parthenogenetic generation had also shown a marked decrease in constitutional vigor.

Investigations of other workers, however, do not seem to confirm these general results. Woodruff, in his observations on the *Paramecium aurelia*, found that a race of these animals kept in cultures made from natural pond waters did not undergo marked depression or physiological changes. This race at the end of eight years, after having passed through 5250 generations without conjugation, was in a normal condition. Another race, however, subjected to the relatively constant hay infusion cultures generally employed, died after passing through several hundred generations.

[1] Studies from the Zoölogical Laboratory, The University of Nebraska, No. 134.

King, also, in experiments with albino rats has found that continued inbreeding of the rat, when subjected to a varied and well balanced diet, does not weaken the race. On the contrary, the strains were larger, more fertile, and lived longer than many strains of stock rats in which no inbreeding was allowed. Castle, also, found after inbreeding the fly *Drosophila* (brother and sister matings) for fifty-nine generations, that no apparent decrease in the size and vigor, as compared to those with which he started, was noticeable. Noyes has recently finished the 250th parthenogenetic generation of the rotifer *Proales decipiens* and has detected no noticeable decline in vigor. Moreover, males of this species have never been seen. East and Jones are inclined to believe that if proper changes in the diet had been made in the experiments of Whitney and Shull upon the rotifer *Hydatina senta* that the normal vigor of the races would have been maintained.

Problem.

The following experiments with the rotifer, *Hydatina senta*, were begun in order to determine if possible whether the gradual loss of vigor is due to the parthenogenetic reproduction or to a too restricted diet. The various races used in these experiments were kept under identical conditions as to temperature, light and culture water. Race *A* was fed a colorless *Polytoma* diet, whereas race *B* was fed a diet consisting of the *Polytoma* plus several kinds of green protozoa, predominately *Chlamydomonas*. The plan has been to keep the races in a parallel series and to study the effect of the two diets upon the two races. A third race (*C*) which has been obtained from wild rotifer cultures in the natural environment has been introduced into the series in order that the rate of reproduction of the rotifers under restricted and controlled environmental conditions might be compared with the rate of reproduction of those under the uncontrolled conditions of nature.

Acknowledgment is due Professor David D. Whitney for his suggestions and supervision of the work.

Methods.

Some dried fertilized eggs of the rotifer, *Hydatina senta*, were collected at Lincoln, Nebraska, on September 17, 1920. One of

these eggs hatched on September 29, and on October 1 two parthenogenetic sisters were isolated. One became the mother of what has been called race *A* and the other became the mother of what has been called race *B*. The two sister parthenogenetic races *A* and *B* were kept in syracuse watch glasses. Usually once in forty-eight hours five daughter-females of each race were isolated, each daughter-female being placed in a separate watch glass. They produced the young females of the succeeding generation. Race *A* was fed on a pure culture of the flagellate, *Polytoma*. This food was grown in horse manure solutions, which was prepared as follows: eight hundred cubic centimeters of fresh horse manure and one thousand cubic centimeters of tap water steam sterilized for one hour. This was cooled and diluted in the proportion of one part horse manure solution to two parts of boiled rain water. *Polytoma* were then put into it in an open pan and placed at about 18° C. The *Polytoma* grew very quickly and in 24–48 hours immense numbers of them were produced. This culture, ranging from 24–96 hours old, was decanted and replenished daily—one part culture medium to two parts boiled rain water being added. From the surface of this culture the *Polytoma* were taken with a sterilized pipette. This food was centrifuged so as to make it concentrated enough so that one drop was sufficient for each watch glass containing a female rotifer and a small amount of filtered rain water.

Race *B* was fed both from the pure culture of *Polytoma* and from two or three cultures of green flagellates, *Chlamydomonas*, *Chloroganium euchlorum*, and *Euglena*.

As some of the green food grew in salty cultures it was always washed with rain water before being placed in the watch glasses as food for the rotifers.

During the whole period of fifteen months the two races have been conducted in parallel generations. The external factors and environment have been as near alike as it has been possible to make them. The individuals of each generation were isolated at the same time, were put into the same kind of watch glasses and with approximately the same amount of filtered tap water, the only difference being in the food. The watch glasses were stacked side by side. During the day the room was lighted by the north light.

The method used for deciding whether the races had maintained their original vitality or whether they had decreased in their constitutional vigor has been the rate of parthenogenetic reproduction. In order to determine the comparative vigor of the races *A* and *B*, their rates of parthenogenetic reproduction were obtained by counting the young produced by a mother during a certain period of time for several successive generations at intervals of about thirty days.

The first comparison was made by counting the young daughter-females of both the races *A* and *B* from generations 22–29. The second comparison was made by counting the young in the same manner as above from the generations 43–47. The third was made by counting the young from generations 61–67. No green food was given to race *B* during the generations when these counts were made so that conditions would be identical at the time the rate of parthenogenetic reproduction was being determined.

During the above named generations fifteen daughter-females of approximately the same size of each race were isolated. This was done in order to give a greater number from which to get the average of young females produced, thereby decreasing the chance of error.

When the third and also the last comparisons were made fifteen females from various wild races of *Hydatina senta* were isolated and placed in separate watch glasses. These wild females have been called race *C*. This race was fed only pure culture of *Polytoma*. It was kept for only a few generations, being conducted under identical conditions and parallel to generations 65–67 and 169–175 of races *A* and *B*. This introduction was made in order to make a comparison of the rate of parthenogenetic reproduction between races *A* and *B* and various wild races taken from their normal environment.

Results.

The first series of observations on the rates of parthenogenetic reproduction in these two races was started at the beginning of the twenty-second generation. The results at this time as can be seen in Table I., are extremely variable. Only five daughter-

TABLE I.

No. of Partheno-genetic Generation.	Date of Isolation.	No. of Young Females Isolated and Reproducing.	Period of Growth and Reproduction.	Race *A*. Av. No. of Daughters Produced.	Race *B*. Av. No. of Daughters Produced.
22.....	11/16/20	5	48 hrs.	9.22	10.25
23.....	11/18/20	5	48	7.20	5
24.....	11/20/20	5	48	4	1
25.....	11/22/20	5	48	7.8	1.4
26.....	11/24/20	5	48	1.5	1.5
27.....	11/26/20	5	72	4.2	4.5
28.....	11/29/20	5	72	13.8	15.66
29.....	12/2 /20	5	48	7	7.2
Average ..		5	54 +	6.84	5.81

females were isolated every other day for making the counts on the two races. The average number of young daughter-females produced in each forty-eight hour period at this time was extremely variable. The mothers of the *A* race during this period produced an average of 6.84 daughter-females while those of the *B* race produced an average of only 5.81.

The two parthenogenetic races were then carried on parallel with each other until the beginning of the forty-third generation at which time another series of observations was started on the number of young daughter-females each mother would produce in a given length of time, approximately 48 hours. As new generations were isolated great care was taken to pick uniform-sized daughters. One investigator did all of the isolating during this series of counts in order to insure the greatest uniformity. Considerable difficulty was experienced during this series of observations because of the large per cent. of male-producing females. These had to be discarded for only the mothers producing daughter-females were considered in calculating the averages.

The *A* race during this second series of observations produced an average of 7.58 daughter-females while the *B* race produced 8.88. Considerable variation occurred throughout the different generations, but the variations were less in this instance than they were in the preceding series of observations. A complete record of the results of this observation can be seen in Table II.

The two races were then carried on for fourteen generations before another series of observations was begun. At the begin-

TABLE II.

No. of Partheno-genetic Generation.	Date of Isolation.	No. of Young Females Isolated and Reproducing.		Period of Growth and Reproduction.	Race *A*. Av. No. of Daughters Produced.	Race *B*. Av. No. of Daughters Produced.
		A.	*B*.			
43.....	1/ 6/21	5	5	48 hrs.	7.4	10
44.....	1/ 8/21	10	11	48	6.6	3.8
45.....	1/11/21	14	13	48	6	10.8
45.....	1/11/21	5	5	48	6.5	6.9
46.....	1/13/21	8	11	48	12	15.2
47.....	1/15/21	15	14	52	7	6.6
Average.....				48.7	7.58	8.88

ning of the 61st generation fifteen individuals from each race were isolated as before and placed in separate watch glasses,—one person doing all the isolating as in the preceding series. No male-producing females appeared in this series of observations and the number of young produced in a given length of time was more nearly uniform throughout than was the case in the two preceding observations. The average number of young produced by the *A* race in the last three generations of this series of counts was 2.66 in an average of 46.3 hours. In the *B* race for the three generations an average of 3.68 daughter-females were produced in the same period of time.

TABLE III.

No. of Partheno-genetic Genera-tion.	Date of Isolation.	No. of Young Females Isolated and Reproducing.			Period of Growth and Repro-duction.	Av. No. of Daughter-Females Produced.		
		A.	*B*.	*C*.		Race *A*.	Race *B*.	Race *C*.
61.....	2/13/21	15	13		48 hrs.	4.84	5.84	
62.....	2/15/21	15	15		41	4.64	5.44	
63.....	2/17/21	15	15		47	2.90	3.37	
64.....	2/19/21	15	15		48	4.25	4.37	
65.....	2/21/21	13	15	15	44	1.15	2	2.33
66.....	2/23/21	15	14	15	48	3.56	4.21	4
67.....	2/25/21	5	14	14	48	3.80	5.21	4.25
Average..					46.3	2.66	3.68	3.53

At the beginning of the sixty-fifth generation, counts on the

wild race of rotifers, race *C*, were started. They were reared under identical conditions with the *A* and *B* races and the counts were carried over a period of three generations. The average number of young produced by these mothers in 46.3 hours was 3.53. A complete record of the results of this observation can be seen in Table III.

The two parthenogenetic races were then carried on parallel with one another until the beginning of the 79th generation at which time a fourth series of observations was started.

During this fourth series of observations the *A* race produced an average of 7.46 daughter-females while the *B* race produced 8.29. Very little variation occurred throughout the different generations during this series of observations. A complete record of the results of this observation is given in Table IV.

TABLE IV.

No. of Parthenogenetic Generation.	Date of Isolation.	No. of Young Females Isolated and Reproducing.		Period of Growth and Reproduction.	Av. No. of Daughter-Females Produced.	
		A.	*B.*		Race *A.*	Race *B.*
79.....	3/22/21	15	15	48 hrs.	10.06	11.46
80.....	3/24/21	15	15	48	7.06	7.53
81.....	3/26/21	13	14	54	3.92	4.28
82.....	3/28/21	15	15	46	6.53	7.26
83.....	3/30/21	15	15	48	9.73	10.93
Average.....				48.8	7.46	8.29

The two races were then carried on for thirteen generations before another series of observations was made. The average number of hours between counts in this series of observations was 65.33 hours, the increase in length of time being due to the slowness of reproduction which was probably caused by the low temperature of the laboratory. The average number of young daughter-females produced during this series by race *A* was 3.32, while the number by race *B* was 5.15. A complete record of the results of this series is given in Table V.

The two races were again carried on parallel with one another until the beginning of the 106th generation. At this time they showed a very great degree of uniformity between the different

TABLE V.

No. of Parthenogenetic Generation.	Date of Isolation.	No. of Young Females Isolated and Reproducing.		Period of Growth and Reproduction.	Av. No. of Daughter-Females Produced.	
		A.	*B.*		Race *A.*	Race *B.*
96.....	4/28/21	14	15	77 hrs.	4.13	6.53
97.....	5/ 1/21	13	15	70	2.23	4.33
98.....	5/ 4/21	15	15	50	3.60	4.60
Average.....				65.33	3.32	5.15

generations, but there was a slight decrease in the difference between the number of young daughter-females produced by the two races. The *A* race during this series of observations produced an average of 9.19 daughter-females while the *B* race produced 10.06. A complete record of the results of this observation is given in Table VI.

TABLE VI.

No. of Parthenogenetic Generation.	Date of Isolation.	No. of Young Females Isolated and Reproducing.		Period of Growth and Reproduction.	Av. No. of Daughter-Females Produced.	
		A.	*B.*		Race *A.*	Race *B.*
106.....	5/23/21	14	13	48 hrs.	9.28	10.07
107.....	5/25/21	14	14	48	8.14	10.64
108.....	5/27/21	15	15	48	6.60	7.20
109.....	5/29/21	15	15	48	11.46	11.46
110.....	5/31/21	13	11	50	10.46	10.91
Average.....				48.4	9.19	10.06

The parthenogenetic reproduction of the parallel races was continued over the summer of 1921 without any further observations. The usual care was employed in their feeding. About the middle of August a wild race, *C*, was introduced. The seventh series of observations was begun with the 169th generation of the *A* and *B* races and the 10th of the wild race *C*. Considerable variation was found in the number of young produced by successive generations of each race. The difference, however, in the number of young of the *B* race as compared to the numbers

of young of *A* and *C* was marked. The average period of growth and reproduction was 63.6 hours, during which period the average number of young of the *A* race was 6.86; of the *B*, 9.35; and of the *C*, 8.66. The complete tabulation of results may be found in Table VII.

TABLE VII.

No. of Parthenogenetic Generation.	Date of Isolation.	Number of Young Females Isolated and Reproducing.	Period of Growth and Reproduction.	Average No. of Daughter-females.		
				A.	*B.*	*C.*
A } *B* } 169..... *C*—10.....	10/ 5/21	10	72 hours	9.4	14.1	14.9
A } *B* } 170..... *C*—11.....	10/ 8/21	10	66 hours	9.8	10.1	10.33
A } *B* } 171..... *C*—12.....	10/11/21	10	56 hours	6.5	9.4	7.9
A } *B* } 174..... *C*—15.....	10/22/21	10	54 hours	1	2.75	1.7
A } *B* } 175..... *C*—16.....	10/25/21	10	70 hours	7.6	10.4	8.5
Averages.....		10	63.6 hours	6.86	9.35	8.66

The three races were then carried on side by side. Since the 145th generation the green food given Race *B* has been made up for the most part of *Euglena*, in place of the *Chlamydomonas* heretofore used. About the same time as the change in the green food the centrifuging of the *Polytoma* culture was discontinued and more drops of the unconcentrated food given instead. After the seventh series of observations, the investigators began to encounter difficulty with the supply of *Polytoma*. However, all three races of rotifers continued to reproduce parthenogenetically, although with slightly less than usual vigor in Race *A*. Nevertheless, notwithstanding all care possible in feeding, isolation and temperature the *A* race, fed only *Polytoma*, died out with the 198th generation, probably due to the faulty

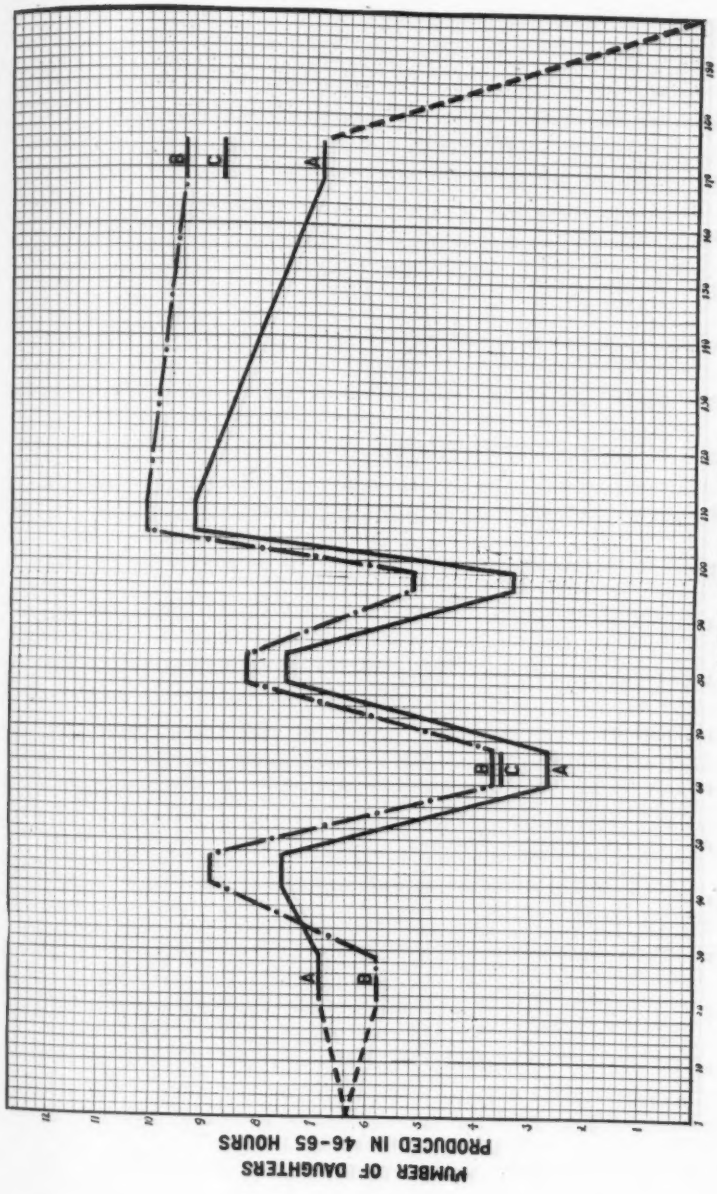

FIG. 1. Graphic representation of the results obtained from parallel races fed upon different diets and reproducing parthenogenetically. The unbroken line traces the history of Race *A*; the broken, of Race *B*; the other, of the wild races *C*, introduced at intervals for comparison. The lines from generation 1 to 22 and 176 to 198 are hypothetical, since no tests were made during these periods. The rate of reproduction of Race *A*, which was kept on the pure *Polytoma* diet, gradually declined, whereas Race *B*, kept on a mixed diet, showed no decline in the rate of reproduction.

Polytoma culture. Race *B* continued to the 209th generation and *C* to the 43rd, without apparent loss of vigor, when the experiment was discontinued. The entire history of the two races, *A* and *B*, may be seen in Fig. 1. These two races started from two sisters and their rate of reproduction was determined at seven different periods ranging over about 13 months. For some unknown reason Race B showed a lower rate of reproduction at the first count, but at the second count the rate had risen above that of Race A, and furthermore, in all the subsequent counts, covering about a year, it remained above the rate of Race A. Toward the end the two rates began to diverge considerably due to the gradual weakening of Race A while Race B maintained its former rate of reproduction.

Conclusions.

From the fact that the *A* race died out before the 200th generation it is obvious that some unexpected influence must have entered, most probably poor food, since other cultures of *Hydatina senta* have reproduced four and five hundred generations parthenogenetically. Therefore, our results can hardly be called conclusive, but the opinion seems to be justified that the constitutional vigor of the *A* race, fed upon an exclusive colorless diet, was noticeably weakened, while that of the *B* race, fed upon a diet of *Polytoma* plus a green food containing carbohydrates, was sustained and even raised above that of the wild races. If this is true, as it certainly seems to be, it would appear that the decline in vigor and eventual death in the races observed by Shull and Whitney was due to an unbalanced diet, rather than to the parthenogenetic method of reproduction, as they concluded.

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